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IgE Enhances Fcε Receptor I Expression and IgE-Dependent Release of Histamine and Lipid Mediators from Human Umbilical Cord Blood-Derived Mast Cells: Synergistic Effect of IL-4 and IgE on Human Mast Cell Fcε Receptor I Expression and Mediator Release¹

Masao Yamaguchi,^{2,3*} Koichi Sayama,^{3*} Koji Yano,^{4*} Chris S. Lantz,* Nancy Noben-Trauth,[†] Chisei Ra,[‡] John J. Costa,*[§] and Stephen J. Galli^{5*}

We investigated the effects of IgE versus IL-4 on FcεRI surface expression in differentiated human mast cells derived in vitro from umbilical cord blood mononuclear cells. We found that IgE (at 5 μg/ml) much more strikingly enhanced surface expression of FcεRI than did IL-4 (at 0.1–100 ng/ml); similar results were also obtained with differentiated mouse mast cells. However, IL-4 acted synergistically with IgE to enhance FcεRI expression in these umbilical cord blood-derived human mast cells, as well as in mouse peritoneal mast cells derived from IL-4^{-/-} or IL-4^{+/+} mice. We also found that: 1) IgE-dependent enhancement of FcεRI expression was associated with a significantly enhanced ability of these human mast cells to secrete histamine, PGD₂, and leukotriene C₄ upon subsequent passive sensitization with IgE and challenge with anti-IgE; 2) preincubation with IL-4 enhanced IgE-dependent mediator secretion in these cells even in the absence of significant effects on FcεRI surface expression; 3) when used together with IgE, IL-4 enhanced IgE-dependent mediator secretion in human mast cells to levels greater than those observed in cells that had been preincubated with IgE alone; and 4) batches of human mast cells generated in vitro from umbilical cord blood cells derived from different donors exhibited differences in the magnitude and pattern of histamine and lipid mediator release in response to anti-IgE challenge, both under baseline conditions and after preincubation with IgE and/or IL-4. *The Journal of Immunology*, 1999, 162: 5455–5465.

Immunoglobulin E- and Ag-dependent activation of mast cell mediator secretion is thought to contribute importantly to the expression of protective immune responses to certain parasites and also to the pathogenesis of asthma and other allergic diseases (1–7). It is now clear that IgE-dependent mast cell effector function in these responses depends almost entirely, if not solely, on the ability of these cells to bind IgE to high affinity IgE receptor (FcεRI) expressed on the surface of cells (8, 9). Moreover, it has long been known that patients with parasite infections or allergic diseases typically have elevated concentrations of IgE (1, 7, 10), and that atopic subjects with high levels of serum IgE also exhibit

increased expression of FcεRI on the surface of blood basophils (11, 12). Yet, until recently, relatively little attention had been given to the possibility that IgE itself might regulate the ability of mast cells (or other FcεRI⁺ effector cells) to bind IgE, or that this might have important implications for mast cell function.

However, it is now clear that IgE can enhance the IgE-binding ability of bone marrow-derived mouse mast cells in vitro (13, 14) and mouse peritoneal mast cells in vitro or in vivo (13), and that this increased IgE-binding ability reflects enhanced cell surface expression of FcεRI (13). The administration of IgE also can increase the expression of FcεRI by rat mast cells in vivo (15). Moreover, such IgE-dependent up-regulation of FcεRI expression can significantly increase the ability of mouse mast cells to release preformed mediators or cytokines in response to challenge with specific Ag (13). IgE-dependent enhancement of mouse mast cell FcεRI expression had a particularly striking effect on the ability of cells to secrete IL-4 (13), a cytokine that can promote the production of IgE (16, 17).

While the relevance of these observations to the human system was not initially clear, we recently reported that IgE can enhance the IgE-binding ability of umbilical cord blood-derived cultured human mast cells, and that such IgE-dependent enhancement of the IgE-binding ability of cells enhances the ability of these anti-IgE-induced mast cells to secrete histamine, and the chemokine macrophage-inflammatory protein-1α (MIP-1α),⁶ in response to challenge with anti-IgE

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⁶ Abbreviations used in this paper: MIP-1α, macrophage-inflammatory protein-1α; BM-CMCs, mouse bone marrow-derived cultured mast cells; h, human; LTC, leukotriene C; MESF, molecules of equivalent soluble fluorochrome units; PMCs, mouse peritoneal mast cells; rhSCF, recombinant human stem cell factor 164; SCF, stem cell factor.

(18). Notably, Kimata et al. (19) have reported recently that MIP-1α can enhance IgE production by human B cells in vitro.

In addition to IgE, IL-4 can also enhance FcεRI expression in certain populations of human mast cells. Toru et al. (20) reported that IL-4 can up-regulate FcεRI expression and IgE binding in human mast cells derived in vitro from CD34⁺ human umbilical cord blood mononuclear cells maintained in stem cell factor (SCF) and IL-6 without PGE₂, and Pawankar et al. (21) found that IL-4 can also enhance surface expression of FcεRI on human nasal mast cells in vitro. While neither of those studies compared the effects of IL-4 versus IgE on human mast cell FcεRI expression, H.-Z. Xia et al. (22) recently did perform such a comparison, using fetal liver-derived human mast cells that had been generated in SCF-containing media in vitro. These fetal liver-derived human mast cells, which developed over a period of 4 wk in vitro in the absence of exogenous rhIL-4, expressed almost no detectable FcεRI on the cell surface (22). In these mast cell populations, Xia et al. found that IL-4 was substantially more potent than IgE alone in inducing the expression of FcεRI, but that IL-4 and IgE had a synergistic effect. Moreover, the ability of IL-4 to enhance FcεRI expression progressively diminished during the first 2 wk of culture, indicating that the cytokine is most effective in inducing FcεRI expression in this system if it is present during the earliest stages of mast cell differentiation (22).

In the present study, we analyzed the effects of IgE versus IL-4 on FcεRI expression in a different population of human mast cells: differentiated mast cells generated in SCF-containing medium from umbilical cord blood mononuclear cells. We find that, in these human mast cells, IgE is substantially more potent than IL-4 in enhancing FcεRI expression, and that IgE-dependent enhancement of FcεRI expression permits the cells to secrete increased amounts of lipid mediators, in addition to histamine, in response to challenge with anti-IgE. However, when added with IgE, IL-4 can enhance mast cell surface expression of FcεRI over that observed in cells that had been preincubated with IgE alone; IL-4 preincubation can also enhance mast cell mediator secretion in response to anti-IgE challenge even without producing significant enhancement of FcεRI surface expression. Some of these results have been reported in abstract form (23).

Materials and Methods

Umbilical cord blood-derived human mast cells

In vitro derived human mast cells were obtained essentially as described in Saito et al. (24). Heparinized cord blood (Advanced Biotechnologies, Columbia, MD, or Brigham and Women's Hospital, Boston, MA) was overlaid on Histopaque 1077 (Sigma, St. Louis, MO) and centrifuged at room temperature for 30 min at 400 × *g*. Mononuclear cells at the interface between plasma and Histopaque 1077 were collected, washed, and then maintained in culture medium, consisting of Iscove's modified Dulbecco's medium (IMDM; Sigma) supplemented with 10% FCS (HyClone, Logan, UT); 80 ng/ml human rSCF¹⁶⁴ (rhSCF; Amgen, Thousand Oaks, CA) (25); 50 ng/ml human rIL-6 (rhIL-6; Amgen); 1 μM PGE₂ (Cayman Chemical, Ann Arbor, MI); and from Life Technologies (Gaithersburg, MD), 10 mM HEPES, 2 mM L-glutamine, antibiotics (100 U penicillin/ml, 100 μg streptomycin/ml, and 10 μg gentamicin/ml), 1 × MEM vitamin solution (catalogue 11120), 1 × MEM amino acids (without L-glutamine, catalogue 11130), 1 mM sodium pyruvate, and 50 μM 2-ME. Half of the culture medium was changed weekly, and cells were cultured for at least 9 wk. The purity of the mast cells at the time of individual experiments was 86 to >99% (as specified in the text), as determined using Kimura's stain (26) and/or tryptase immunostaining (24); the two methods usually gave essentially identical results. In some experiments, mast cells were incubated (unless specified otherwise, in the usual culture medium) for up to 8 days with or without purified human myeloma IgE (Bioscience International, Kennebunk, ME, at 0.05 or 5 μg/ml microcentrifuged at ~14,000 × *g* for 20 min at 20°C to remove possible aggregates), and/or rhIL-4 (Genzyme, Cambridge, MA), before analyses of mast cell surface FcεRI expression and/or mediator release (see below). After centrifugation, IgE was removed

from the top 20% of the solution for incubation with mast cells; the IgE concentrations of solutions before or after centrifugation were 104.6 ± 2.6% or 101.2 ± 2.6% of the supplier's reported values, and the difference between these values was not statistically significant. Mast cell numbers and viability (which was always >90% according to trypan blue staining) were assessed immediately before analysis of mast cell FcεRI surface expression or mediator release (i.e., after culture and/or preincubation for up to 8 days ± IgE or IL-4).

Flow cytometry of umbilical cord blood-derived human mast cells

Cells were washed once in DMEM (Life Technologies) supplemented with 3% FCS (Sigma), then preincubated with human IgG (Sigma) at 10 μg/ml for 15 min, and then incubated at 4°C with purified human myeloma IgE (Bioscience International) at 10 μg/ml for 50 min; pilot studies showed that these conditions resulted in ~96% of the level of binding of IgE to mast cells as was obtained with cells that had been incubated with IgE at 50 μg/ml for 150 min at 4°C. After washing once in DMEM supplemented with 3% FCS (Sigma), cells were stained with FITC goat anti-human IgE Ab (Bioscience International, Camarillo, CA; at 9 μg/ml) for 25 min at 4°C. To analyze directly levels of mast cell surface FcεRI expression, cells were preincubated with human IgG for 15 min (as above) and then incubated for 1 h at 4°C with 5 μg/ml of the mouse IgG2b anti-human FcεRI α-chain mAb, CRA-1, which can bind to the FcεRI α-chain whether or not it is occupied by IgE (21, 27). After washing, cells were stained with FITC goat F(ab')₂ against mouse IgG (Jackson ImmunoResearch, West Grove, PA) at 7.5 μg/ml for 30 min. An isotype-matched mouse IgG2b mAb with irrelevant specificity (MOPC195; Organon Teknica, Durham, NC) was used as negative control instead of CRA-1. Stained cells were analyzed using a FACSCalibur (Becton Dickinson, San Jose, CA). Ten thousand events in each sample were analyzed, and at least five thousand mast cells were studied to calculate the median value of fluorescence intensity.

An instrument setting of the flow cytometer that was appropriate for analysis of human mast cells was determined in the first experiment and was used for all such analyses throughout this study. In addition, the median values of fluorescence intensity of mast cells were converted to the numbers of the molecules of equivalent soluble fluorochrome units (MESF) using Quantum 25 microbeads (Flow Cytometry Standards, San Juan, PR) on each day that an experiment was performed, as per the specifications of the manufacturer (13).

Studies of differentiated mouse mast cells

For cell culture before flow cytometry, unfractionated peritoneal cells from adult BALB/c IL-4^{-/-} mice (28) or the corresponding normal (IL-4^{+/+}) BALB/c mice (Charles River Laboratory, Wilmington, MA) were cultured at 5 × 10⁵ cells/ml in DMEM (Life Technologies) supplemented with 10% FCS, 50 ng/ml rat rSCF (Amgen), 2 mM L-glutamine, and antibiotics (penicillin/streptomycin); mast cells were incubated for up to 6 days with or without ascites containing mouse IgE anti-DNP mAb (at 5 μg/ml) (13) or mouse rIL-4 (Genzyme; at 10 ng/ml).

BALB/c bone marrow-derived cultured mast cells (BMCMCs) were generated by culturing femoral bone marrow cells of adult BALB/c mice in 20% WEHI-3 cell supernatant-conditioned medium for 4–6 wk. Mast cells were incubated for up to 4 days with or without ascites containing mouse IgE anti-DNP (at 5 μg/ml) or mouse rIL-4 (at 10 ng/ml).

For flow-cytometric analysis of unfractionated freshly isolated mouse peritoneal mast cells, we preincubated the cells with B3B4 and 2.4G2 mAbs (PharMingen, San Diego, CA) for 15 min to block low affinity binding of IgE or other subsequent Abs to CD23 or FcγRII/III, respectively (13). The cells were then incubated at 4°C with mouse IgE anti-DNP mAb (at 10 μg/ml) for 50 min, and then, simultaneously, for the last 25 min, with a biotinylated rat anti-mouse c-kit Ab (at 15 μg/ml) (13). After washing, cells were stained with FITC rat anti-mouse IgE Ab (PharMingen; at 10 μg/ml) and phycoerythrin-streptavidin (Sigma; at 14 μg/ml) for 25 min at 4°C. IgE⁺, c-kit⁺ cells were gated and analyzed (13).

For flow-cytometric analysis of BMCMCs, cells were blocked with B3B4 and 2.4G2 as above, incubated with mouse IgE anti-DNP mAb (at 10 μg/ml) for 50 min, and then stained with FITC rat anti-mouse IgE Ab (at 10 μg/ml) (13).

Quantification of human mast cell mediator release

For measurements of mediator release, cells were sensitized with myeloma IgE at 5 μg/ml for 2 h at 37°C, washed, and stimulated with either goat anti-human IgE Ab or calcium ionophore A23187 (Sigma). For measurements of histamine, PGD₂, or LTC₄ release, cells were stimulated for 1 h at 37°C, and the histamine in supernatant and cell fractions was measured

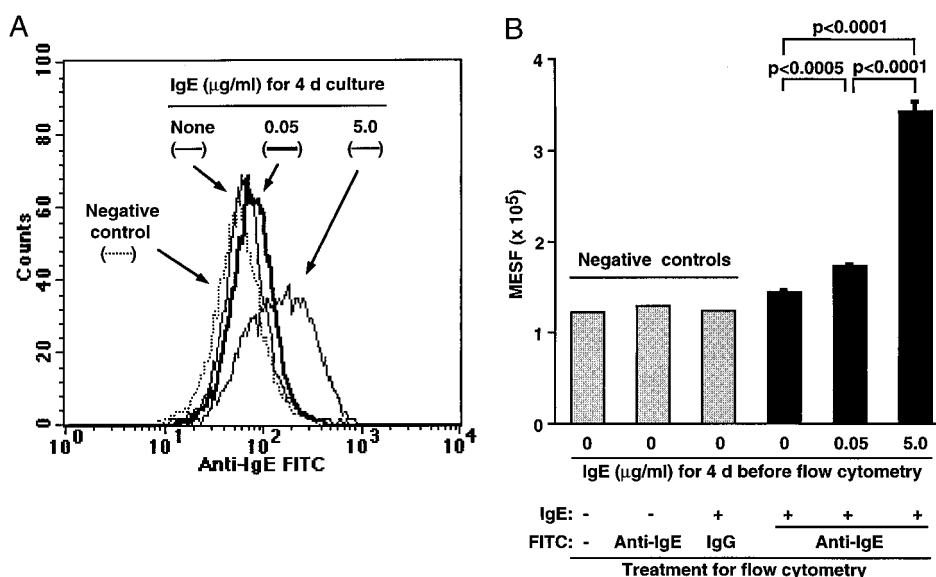


FIGURE 1. A and B, Up-regulation of umbilical cord blood-derived human mast cell IgE binding by IgE. Mast cells (purity = 98%, 19 wk of culture) were cultured without IgE or with purified human myeloma IgE at 0.05 or 5 µg/ml for 4 days, washed, preincubated for 15 min with human IgG (10 µg/ml), and then incubated with excess IgE (10 µg/ml) at 4°C for 50 min to saturate fully mast cell surface FcεRI, and then mast cell surface IgE binding was analyzed by flow cytometry. A, Flow cytometry results (negative control = results for mast cells that had been cultured without IgE for 4 days and then stained with FITC goat IgG against human IgE (FITC anti-IgE) with no prior sensitization with IgE). B, The median values of fluorescence intensity of mast cells as shown in A were converted to the numbers of MESF (see *Materials and Methods*). Data for cells stained for surface IgE binding are mean ± SEM ($n = 3$). The three negative controls ($n = 1$ each) show values for cells cultured for 4 days without IgE before preparation for flow cytometry after either omission of FITC anti-IgE and/or IgE, or substitution of FITC goat IgG for FITC goat IgG against human IgE (as indicated in the figure).

using a RIA kit (Immunotech, Westbrook, ME), whereas the PGD₂ or LTC₄ in the supernatants were measured using enzyme immunoassay kits (Cayman Chemical, Ann Arbor, MI). For cytokine release, cells were stimulated with anti-IgE for 4 h, and IL-13 in supernatants was assayed using an ELISA kit (Endogen, Woburn, MA).

Statistical analysis

Unless otherwise specified, all data are expressed as mean ± SEM, and all differences between values were compared by the two-tailed Student's *t* test (unpaired).

Results

IgE enhances FcεRI expression by human mast cells in vitro

As assessed by flow cytometry (see *Materials and Methods*), we confirmed that baseline levels of IgE binding by human mast cells were significantly enhanced, in a concentration-dependent manner, upon incubation with monomeric human IgE (18); results representative of those obtained in 12 separate experiments, employing mast cells derived from eight different umbilical cord blood preparations that were tested after 9–19 wk of culture, are shown in Fig. 1, A and B.

Under the conditions of our experiments, the IgE binding that we assess by flow cytometry reflects, largely, if not entirely, the binding of IgE to FcεRI (13). However, to investigate this point directly, we incubated in vitro derived human mast cells (purity = 96.3%, 9 wk of culture) for 6 days with or without IgE (at 5 µg/ml) and then used flow cytometry to compare the IgE-binding ability of cells, as determined by staining with our FITC anti-human IgE Ab, with their surface expression of FcεRI, as assessed by staining with the CRA-1 Ab (27). As shown in Fig. 2, preincubation of mast cells for 6 days with IgE markedly up-regulated both the ability of these cells to bind IgE and their reactivity with CRA-1. These results indicate that, most, if not all, of the IgE-dependent up-regulation of IgE binding exhibited by these cells reflected increased surface expression of FcεRI.

Comparison of the effects of IgE, IgG, IL-4, or IgE plus IL-4 on FcεRI expression by human mast cells in vitro

We maintained aliquots of some of the same mast cells shown in Fig. 1 for 7 days in medium containing rhSCF, rhIL-6, and 1 µM PGE₂ (i.e., our usual culture medium), or in medium containing the same concentrations of rhSCF and rhIL-6, but without PGE₂

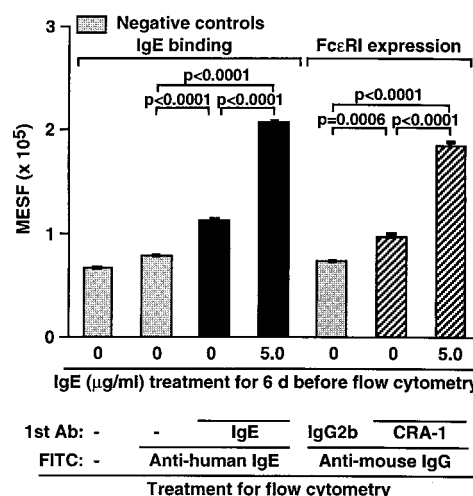


FIGURE 2. Comparison of the surface IgE-binding and FcεRI expression of umbilical cord blood-derived human mast cells. Mast cells (purity = 96.3%, 9 wk of culture) were cultured for 6 days in our usual culture medium with or without human myeloma IgE (5 µg/ml). IgE binding was analyzed by flow cytometry with FITC anti-IgE (as in Fig. 1), and FcεRI expression was analyzed by flow cytometry with the mouse IgG2b mAb CRA-1, which can bind to the FcεRI α-chain whether or not the receptor is occupied by IgE (see *Materials and Methods*). Data are mean ± SEM ($n = 3$).

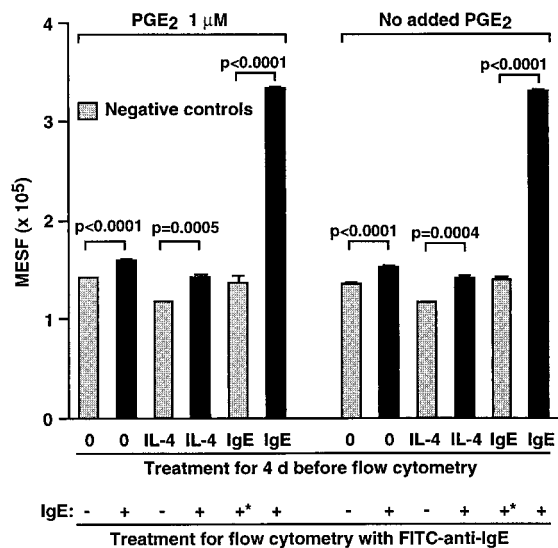
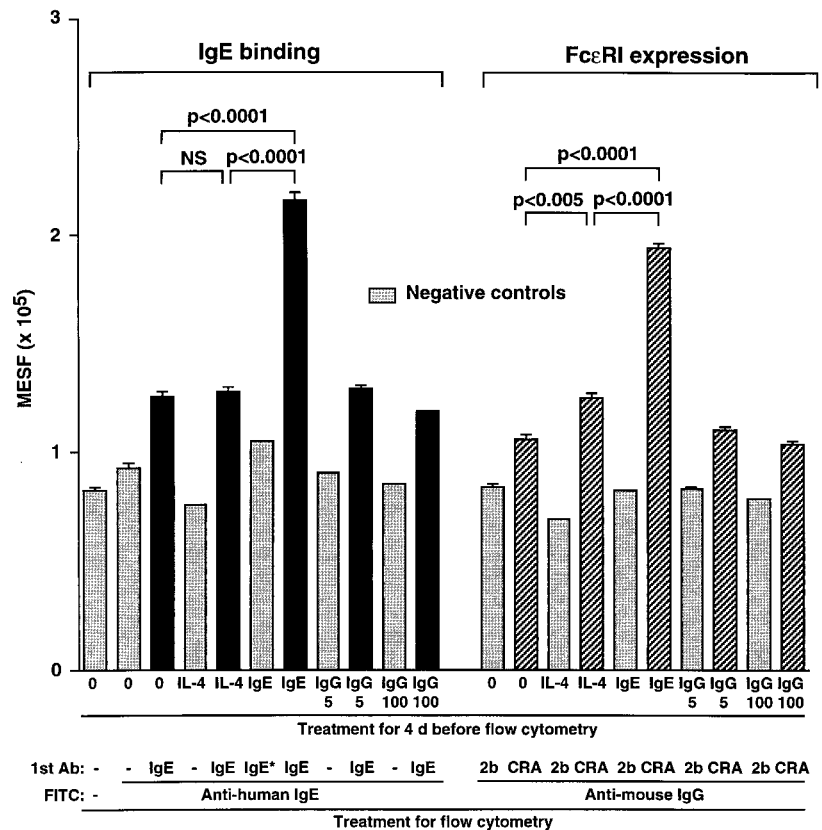


FIGURE 3. Comparison of the effects of IL-4 and IgE on surface IgE binding of umbilical cord blood-derived human mast cells. Mast cells (purity = 98%, 17 wk of culture) were washed and then maintained for 11 days before flow cytometry in the usual culture medium (containing rhSCF, rhIL-6, and PGE₂) or in medium that differed from the usual culture medium only by lacking added PGE₂ (as shown in the figure); the media were supplemented during the last 4 days of culture with rhIL-4 (10 ng/ml), human myeloma IgE (5 μg/ml), or neither IL-4 nor IgE (0 in the figure). IgE binding was analyzed by flow cytometry as in Fig. 1. Negative controls for cells cultured for 4 days ± IL-4, but without IgE, were performed by incubating cells with FITC anti-IgE without any prior sensitization with IgE. Negative controls for cells cultured with IgE for 4 days (+* in the figure) were further sensitized with IgE, washed, and then incubated with excess non-FITC-labeled IgE (at 100 μg/ml) for 10 min before adding FITC anti-IgE (at 9 μg/ml). Data shown are mean ± SEM (*n* = 3).

FIGURE 4. Comparison of the effects of IgE, IL-4, and IgG on surface IgE-binding and surface FcεRI expression in umbilical cord blood-derived human mast cells. Mast cells (purity = 96.3%, 10 wk of culture) were cultured for 4 days in the usual culture medium with added human myeloma IgE (at 5 μg/ml), rhIL-4 (at 10 ng/ml), human IgG (at 5 or 100 μg/ml), or neither Abs nor exogenous IL-4 (0 in the figure). Surface IgE-binding and FcεRI expression were analyzed by flow cytometry as in Fig. 2. Negative controls for IgE binding of cells cultured with IgE for 4 days (IgE* in the figure) were further sensitized with IgE, washed, and then incubated with excess non-FITC-labeled IgE for 10 min before adding FITC anti-IgE (as in Fig. 3). CRA denotes the CRA-1 mouse IgG2b mAb with specificity for the human FcεRI α-chain, whereas 2b denotes a mouse IgG2b mAb with irrelevant specificity (MOPC195).



(20), and then for an additional 4 days in medium containing rhSCF and rhIL-6 ± PGE₂ (1 μM) plus human rIL-4 (Genzyme; at 10 ng/ml) (20, 21), human IgE (as above, at 5 μg/ml), or neither rhIL-4 nor IgE.

As shown in Fig. 3, incubation of mast cells with monomeric IgE (in the presence or absence of exogenous PGE₂) resulted in a substantial enhancement of the IgE-binding ability of cells. When compared with the most appropriate negative control cells (i.e., cells that had not been passively sensitized with IgE immediately before flow cytometry with FITC anti-IgE, but that had been incubated under the same culture conditions for the previous 4 days), the mast cells that had been incubated with rhIL-4 expressed a slightly greater ability to bind IgE (i.e., a somewhat greater difference in the height of the black and grey bars in the figure) than did mast cells that had been incubated without rhIL-4 (Fig. 3). However, any effect that IL-4 may have had on mast cell surface expression of FcεRI in this experiment was minimal compared with that of IgE.

In a second experiment, which employed cells that had been maintained throughout the study in our usual culture medium (i.e., with PGE₂ at 1 μM), we assessed both the IgE-binding ability and the FcεRI expression of mast cells that had been incubated for 4 days before flow cytometry with rhIL-4 (10 ng/ml), monomeric IgE (5 μg/ml), human IgG (Reagent Grade, Sigma; at 5 or 100 μg/ml), or neither Abs nor rhIL-4 (Fig. 4). As assessed by comparison with the most appropriate negative control cells (grey bars in Fig. 4), mast cells that had been incubated with rhIL-4 exhibited slightly enhanced IgE binding and FcεRI expression (Fig. 4). Moreover, the MESF values for FcεRI expression (i.e., CRA-1 binding) of cells incubated with rhIL-4 were significantly higher, by ~18%, than those for cells incubated without rhIL-4, *p* < 0.005 (Fig. 4). However, the effects of rhIL-4 on the ability of the cells to bind IgE, or their surface expression of FcεRI, were much less

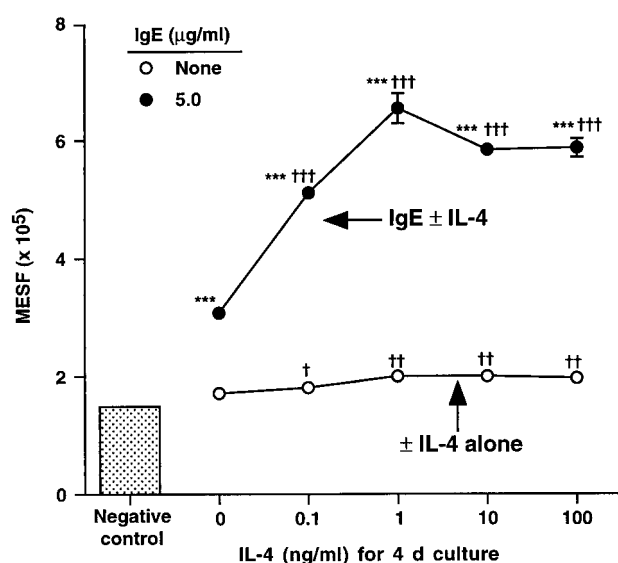


FIGURE 5. Dose-dependent effects of IL-4, with or without IgE, on surface FcεRI expression in umbilical cord blood-derived human mast cells. Mast cells (purity = 100%, 15 wk of culture) were cultured for 4 days in the usual culture medium with or without human myeloma IgE (at 5 μg/ml) and/or rhIL-4 (at 0.1, 1, 10, or 100 ng/ml). Surface FcεRI expression was analyzed by flow cytometry using CRA-1 as in Fig. 2. Negative control (bar) shows values for cells cultured for 4 days without IgE or IL-4 before preparation for flow cytometry, but with omission of staining with FITC anti-mouse IgG. Data are mean ± SEM ($n = 3$); ***, $p < 0.0001$, versus MESF values of corresponding cells that had been maintained for 4 days in the same concentration of IL-4, but without IgE; †, $p < 0.05$; ††, $p < 0.005$; †††, $p < 0.0001$, versus MESF values of cells that had been cultured for 4 days with the same concentration of IgE (i.e., 0 or 5 μg/ml), but without IL-4.

striking than those of IgE ($p < 0.0001$ for either comparison). By contrast, incubation with IgG (at 5 or 100 μg/ml) had no detectable effect on the FcεRI expression of the cells (Fig. 4).

We then analyzed the effects of various concentrations of IL-4 on FcεRI expression, using mast cells maintained in our usual culture medium (with PGE₂, at 1 μM) and cultured for 4 days with or without IL-4 and/or IgE (at 5 μg/ml) (Fig. 5). As assessed by flow cytometry, MESF values for cells cultured for 4 days with IL-4 alone (at 0.1–100 ng/ml) were slightly but significantly higher than values for cells cultured without IL-4, with the greatest enhancement of FcεRI expression by IL-4 alone occurring in cells that had been incubated with 10 ng/ml of IL-4. By contrast, IgE alone (at 5 μg/ml) had a much greater effect on FcεRI expression than did any of the tested concentrations of IL-4 (Fig. 5). However, when various concentrations of IL-4 were added for the 4-day period of culture together with IgE at 5 μg/ml, IL-4 clearly exhibited a concentration-dependent synergistic enhancement of FcεRI expression compared with that induced by IgE alone (Fig. 5).

We then examined the time courses of these effects, using a different batch of mast cells. As shown in Fig. 6, in this experiment IL-4 had little or no effect on FcεRI expression when used without IgE, but significantly enhanced FcεRI expression, over that seen in cells that had been incubated with IgE alone, when administered together with IgE. This synergistic effect of IL-4 was statistically significant as early as 12 h after the beginning of the experiment, and surface FcεRI expression in cells cultured with IgE ± IL-4 continued to increase gradually with time over the 8-day period of the experiment.

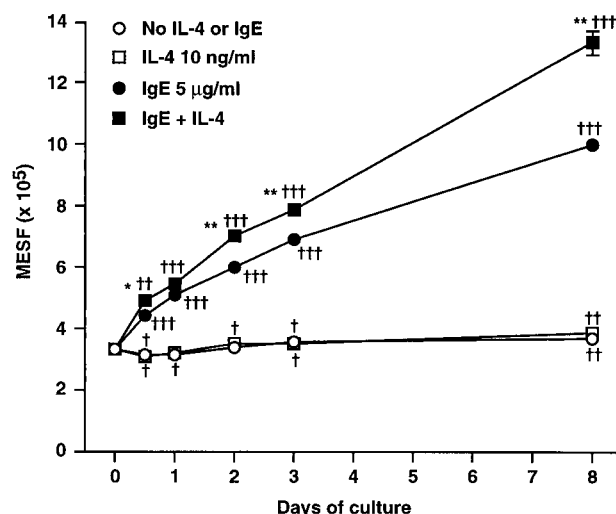


FIGURE 6. Time course of changes in surface FcεRI expression in umbilical cord blood-derived human mast cells cultured with or without IgE and/or IL-4. Mast cells (purity = 92.6%, 14 wk of culture) were cultured in the usual culture medium with or without human myeloma IgE (at 5 μg/ml) and/or rhIL-4 (at 10 ng/ml). Surface FcεRI expression was analyzed by flow cytometry using CRA-1 as in Fig. 2. All data are mean ± SEM ($n = 3$); *, $p < 0.05$; **, $p < 0.005$; ***, $p < 0.0001$, versus MESF values of corresponding cells that had been maintained at the same concentration of IgE (i.e., 0 or 5 μg/ml), but without IL-4; †, $p < 0.05$; ††, $p < 0.005$; †††, $p < 0.0001$, versus MESF value at day 0.

We now have compared the effects of IL-4 versus IgE on IgE-binding ability and/or FcεRI surface expression in eight different batches of human umbilical cord blood-derived mast cells. Preincubation with IL-4 alone increased IgE-binding ability and/or FcεRI expression significantly, albeit minimally, in four of these experiments; in the other four experiments, no significant effects were detected. By contrast, IgE preincubation resulted in marked elevations of IgE-binding ability and/or FcεRI surface expression in all eight experiments.

IgE-dependent up-regulation of human mast cell FcεRI expression can enhance IgE-dependent mast cell release of histamine and lipid mediators, whereas IL-4 can enhance anti-IgE-dependent mediator release without affecting levels of surface expression of FcεRI

We also assessed the potential effects of IgE-dependent up-regulation of IgE binding (Fig. 7A) on the ability of cells to release histamine upon stimulation with either anti-IgE or the FcεRI-independent agonist, the calcium ionophore A23187 (Fig. 7B). In confirmation of our previous findings (18), we found that, in comparison with cells that had not been preincubated with IgE, cells that had been preincubated for 4 days with IgE at 5 μg/ml exhibited an FcεRI-dependent histamine release response to anti-IgE challenge that was significantly enhanced in both sensitivity and intensity; by contrast, the histamine release response to challenge with A23187 was not significantly affected (Fig. 7B). In a separate experiment, which was performed using aliquots of the same mast cell preparations shown in Fig. 1B, we found that the cells that had been incubated in 5 μg of IgE/ml for 4 days gave 73% more specific histamine release upon challenge with anti-IgE at 1 μg/ml than did cells that had not been incubated with IgE before passive sensitization and anti-IgE challenge.

We next performed preliminary experiments that indicated that 1 h was a suitable period at which to measure PGD₂ or LTC₄ release after FcεRI-dependent activation of human umbilical cord

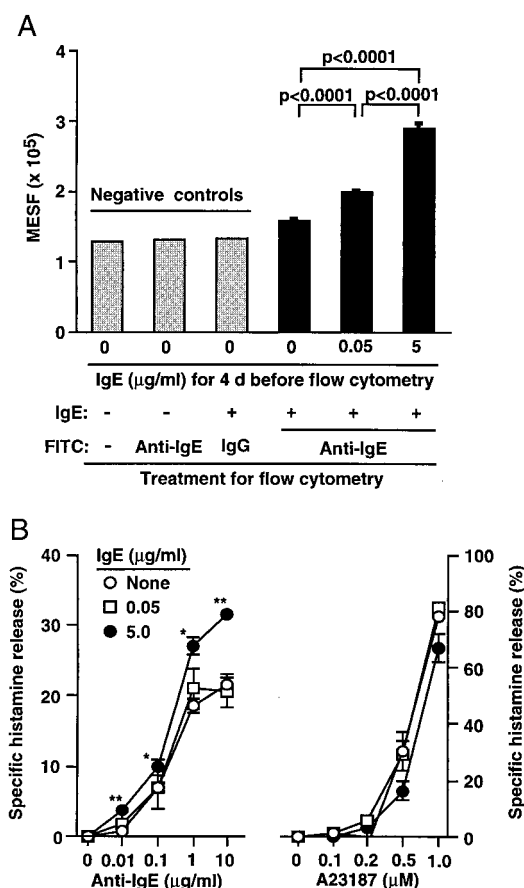


FIGURE 7. Surface IgE-binding ability (A) and specific histamine release (B) of human umbilical cord blood-derived mast cells (purity = 98.3%, 16 wk of culture) after 4 days in culture with IgE at 0, 0.05, or 5 $\mu\text{g/ml}$. A, For flow cytometry analysis of surface IgE binding, cells were briefly incubated with excess IgE and stained with FITC anti-IgE, as in Fig. 1; data shown at mean $\pm$ SEM ($n = 3$). Negative controls were as described in Fig. 1B. B, Mast cells were cultured with or without human IgE for 4 days, washed, and then passively sensitized with IgE at 5 $\mu\text{g/ml}$ for 2 h, and then challenged with various concentrations of anti-IgE or calcium ionophore A23187 for 1 h; all data are mean $\pm$ SEM ($n = 3$). The percentage of specific histamine release was calculated by subtracting baseline (control) release in the absence of anti-IgE (always $\leq 10\%$). *, $p \leq 0.05$; **, $p < 0.005$, versus corresponding values for mast cells that had been cultured for 4 days without IgE.

blood-derived mast cells (data not shown). We then assessed LTC₄ production 1 h after FcεRI-dependent activation in a batch of mast cells (purity >90%, 10 wk of culture) that either had or had not been preincubated with 5 μg IgE/ml for 4 days before challenge with anti-IgE. In this experiment, IgE preincubation resulted in a 47% increase ($p < 0.0001$) in the MESF values of cells assessed after incubation with the CRA-1 Ab to the FcεRI α -chain, and a 156% increase ($p < 0.0001$) in LTC₄ production by 1 h after challenge with anti-IgE at 1 $\mu\text{g/ml}$. Thus, cells that had not been preincubated for 4 days with IgE released 0 ± 0 versus 16.7 ± 1.5 pg LTC₄/10⁴ cells after challenge with 0 versus 1 $\mu\text{g/ml}$ of anti-IgE ($p < 0.01$), whereas the cells that had been preincubated with IgE for 4 days released 0 ± 0 versus 59.5 ± 0.8 pg LTC₄/10⁴ cells ($p < 0.0001$).

We next tested a batch of mast cells of purity >99% (15 wk of culture), to examine the effect of preincubation with IL-4 and/or IgE on the FcεRI expression of cells, and on their ability to release histamine, LTC₄, and PGD₂ in response to FcεRI-dependent activation (Fig. 8). In confirmation of our other experiments, a 4-day

preincubation with IL-4 alone did not significantly enhance FcεRI expression in these cells, whereas FcεRI expression was significantly increased by preincubation with IgE at 5 $\mu\text{g/ml}$ or, to a slightly but significantly greater extent, by preincubation with both IgE and IL-4 (Fig. 8A).

In the same population of cells, preincubation with IgE or IL-4 enhanced anti-IgE-dependent histamine release to a similar extent, whereas the greatest release was observed in cells that had been preincubated with both IgE and IL-4 (Fig. 8B, left). Notably, a 4-day preincubation of these cells with IL-4 at 10 ng/ml also significantly enhanced their ability to release histamine in response to challenge with the calcium ionophore A23187 (1 μM), whereas the A23187-induced responses of cells preincubated with IgE alone were slightly, but not significantly, less than those of control cells that had not been preincubated with IL-4 or IgE (Fig. 8B, right). Since preincubation of these cells with IL-4 had little or no effect on their level of surface expression of FcεRI (Fig. 8A), the effect of IL-4 preincubation on the histamine release response of the cells to challenge with A23187 must have occurred by a mechanism independent of those related to increased surface expression of the FcεRI.

In accordance with the results for histamine release, preincubation of mast cells with either IL-4 or IgE for 4 days enhanced the ability of cells to release LTC₄ or PGD₂ in response to anti-IgE challenge (Fig. 8C). Moreover, for either of these lipid mediators, cells that had been preincubated for 4 days with both IL-4 and IgE released significantly more product in response to anti-IgE challenge than did cells that had been preincubated with either IL-4 or IgE alone.

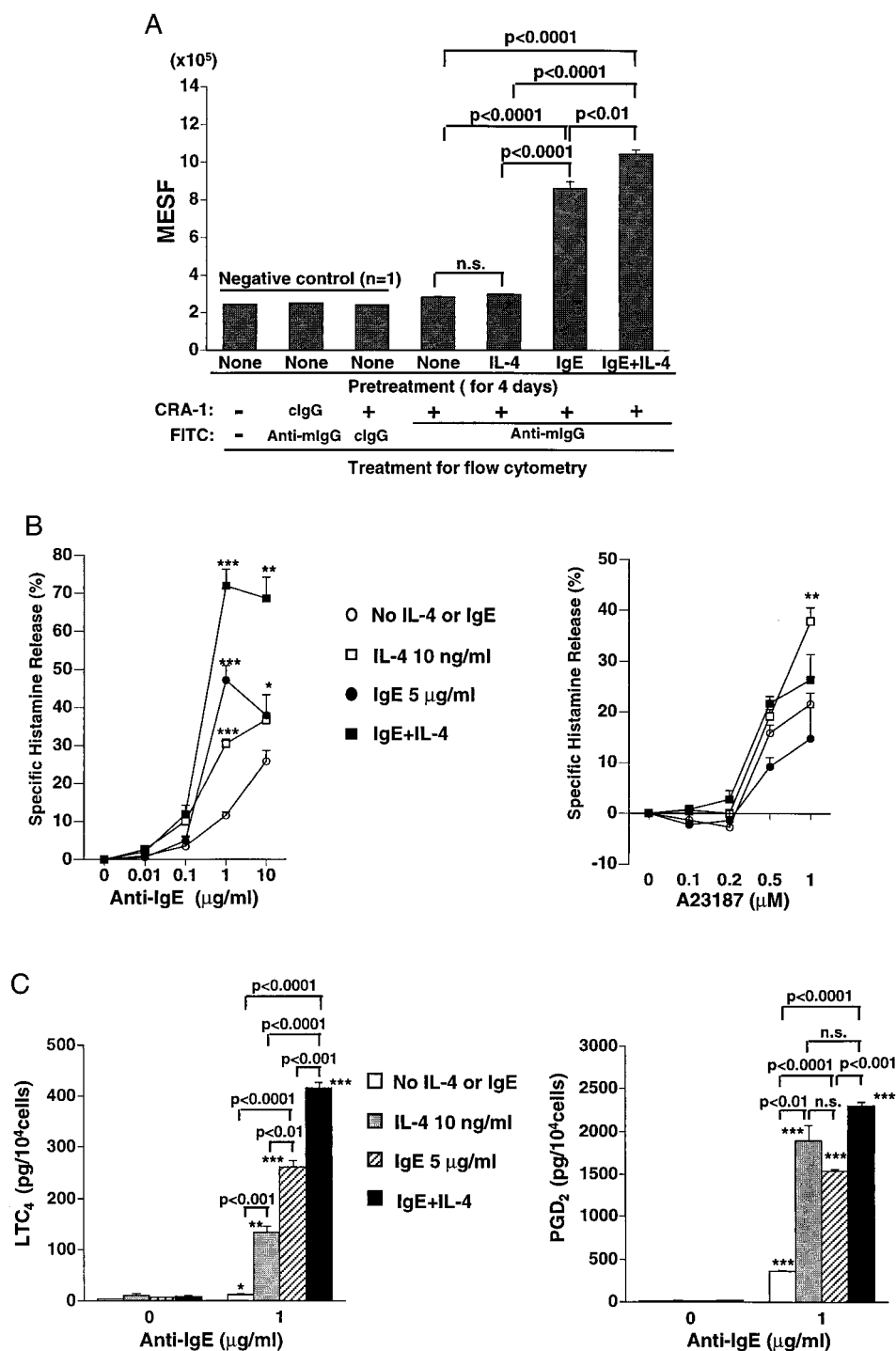
Finally, the data in Fig. 8 indicate that exposure of human umbilical cord blood-derived mast cells to IL-4 can enhance the ability of these cells to release lipid mediators (Fig. 8C) in response to anti-IgE challenge by a mechanism that appears to be independent of any major effect of IL-4 on the cells' surface expression of FcεRI (Fig. 8A). Thus, pretreatment with IL-4 alone had no significant effect on the cells' surface expression of FcεRI (Fig. 8A), but did significantly enhance the ability of cells to release LTC₄ (Fig. 8C, left) or PGD₂ (Fig. 8C, right), as well as histamine (Fig. 8B), upon anti-IgE-dependent activation.

Different batches of umbilical cord blood-derived human mast cells can vary markedly in their mediator release responses to anti-IgE challenge after preincubation with or without IgE and/or IL-4

We then performed an experiment similar to that depicted in Fig. 8, but employing a different batch of cord blood-derived mast cells and including a dose-response examination of the effects of various concentrations of anti-IgE on LTC₄ and PGD₂ release, as well as on histamine release (Fig. 9). In this experiment, preincubation with IL-4 alone slightly, but significantly, enhanced surface expression of FcεRI, whereas preincubation with IgE alone had a much greater effect (Fig. 9A). However, the synergistic effect of IL-4 and IgE preincubation of FcεRI surface expression was greater in these mast cells than in those that were employed for the studies shown in Fig. 8 (cf Figs. 8A and 9A).

Moreover, the mast cells that were used for the experiments in Fig. 9 appeared to be substantially less responsive to anti-IgE-induced release of histamine or lipid mediators than were the cells used for the experiments shown in Fig. 8. For example, minimal specific release of histamine, LTC₄, and PGD₂ (white circles in Fig. 9, B and C, respectively) was induced by anti-IgE challenge of cells that were preincubated with neither IgE nor IL-4 before short-term incubation with IgE before anti-IgE challenge. However, the anti-IgE-induced release of all three mediators was significantly

FIGURE 8. Surface FcεRI expression (A), specific histamine release (B), and LTC₄ and PGD₂ secretion (C) of human umbilical cord blood-derived mast cells (purity >99%, 15 wk of culture) after 4 days in culture with or without IgE at 5 μg/ml and/or IL-4 at 10 ng/ml. A, For flow cytometry analysis of surface IgE expression, cells were incubated with CRA-1 anti-α-chain mAb and stained with FITC anti-mouse IgG; data shown are mean ± SEM (*n* = 3). Negative controls were as described in Fig. 1B. The clgG denotes the isotype-matched control IgG. B and C, Mast cells were cultured with or without IgE at 5 μg/ml and/or IL-4 at 10 ng/ml for 4 days, washed, and then passively sensitized with IgE at 5 μg/ml for 2 h, and then challenged with various concentrations of anti-IgE or calcium ionophore A23187 for 1 h; all data are mean ± SEM (*n* = 3). B, The percentage of specific histamine release was calculated by subtracting baseline (control) release in the absence of anti-IgE. ○, No pretreatment; □, IL-4 at 10 ng/ml; ●, IgE at 5 μg/ml; ■, both IgE at 5 μg/ml and IL-4 at 10 ng/ml. *, *p* < 0.05; **, *p* < 0.001; ***, *p* < 0.0001, versus corresponding values for mast cells that had been cultured for 4 days without IgE or IL-4. C, Left, LTC₄ secretion; right, PGD₂ secretion. White bars, no pretreatment; stippled bars, IL-4 at 10 ng/ml; striped bars, IgE at 5 μg/ml; black bars, both IgE at 5 μg/ml and IL-4 at 10 ng/ml. *, *p* < 0.05; **, *p* < 0.001; ***, *p* < 0.0001, versus corresponding values for mast cells of the same pretreatment group, but that had not been stimulated with anti-IgE.



increased, versus values for cells preincubated without IgE or IL-4, in cells that had been preincubated with either IgE or IL-4 alone, and the enhancing effects of the preincubation with IL-4 alone were the same as (for PGD₂ release, Fig. 9C) or even greater than (for histamine, Fig. 9B, or LTC₄, Fig. 9C) those observed with cells that were preincubated with IgE alone. The enhancing effects, on anti-IgE-induced mediator secretion, of preincubation with both IL-4 and IgE were greater in the cells shown in Fig. 9 than in those shown in Fig. 8, particularly in comparison with the effects observed in cells that were incubated with IgE alone.

Analysis of results obtained with several different batches of umbilical cord blood-derived human mast cells provides further support for the conclusion that such cells can exhibit considerable

batch-to-batch variation in the magnitude of their mediator release responses to anti-IgE challenge, as well as in their patterns of responsiveness to anti-IgE challenge after preincubation with or without IgE and/or IL-4. Thus, we have now analyzed the effects of preincubation with IgE alone on anti-IgE-induced histamine release in two experiments, and have compared the effects of preincubation with IgE alone, IL-4 alone, or both IgE and IL-4 on anti-IgE-induced histamine release in five experiments, for a total of seven experiments, each employing a different batch of cord blood-derived mast cells. In two of these experiments (those depicted in Figs. 7 and 8), anti-IgE challenge induced >10% specific histamine release in cells that had been preincubated with neither IgE nor IL-4, whereas in five experiments (including that depicted

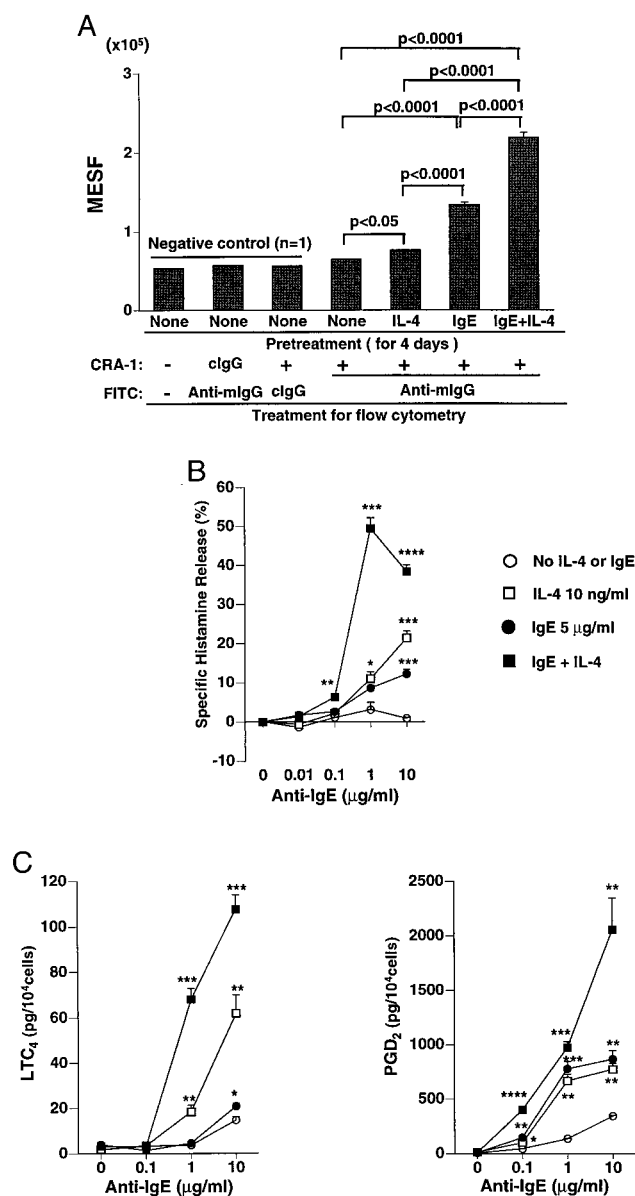


FIGURE 9. Surface FcεRI expression (A), specific histamine release (B), and LTC₄ and PGD₂ secretion (C) of human umbilical cord blood-derived mast cells (purity 98.7%, 11 wk of culture) after 4 days in culture with or without IgE at 5 μg/ml and/or IL-4 at 10 ng/ml. A, For flow cytometry analysis of surface FcεRI expression, cells were incubated with CRA-1 anti-α-chain mAb and stained with FITC anti-mouse IgG; data shown are mean ± SEM (n = 3). Negative controls were as described in Fig. 1B. The clgG denotes the isotype-matched control IgG. B and C, Mast cells were cultured with or without IgE at 5 μg/ml and/or IL-4 at 10 ng/ml for 4 days, washed, and then passively sensitized with IgE at 5 μg/ml for 2 h, and then challenged with various concentrations of anti-IgE for 1 h; all data are mean ± SEM (n = 3). Open circles, no pretreatment; open squares, IL-4 at 10 ng/ml; solid circles, IgE at 5 μg/ml; solid squares, both IgE at 5 μg/ml and IL-4 at 10 ng/ml. *, p < 0.05; **, p < 0.01; ***, p < 0.001; ****, p < 0.0001, versus corresponding values for mast cells that had been cultured for 4 days without IgE or IL-4. B, The percentage of specific histamine release was calculated by subtracting baseline (control) release in the absence of anti-IgE. C, Left, LTC₄ secretion; right, PGD₂ secretion.

in Fig. 9), anti-IgE challenge induced <10% specific histamine release from such control cells.

Although we have performed relatively few studies of anti-IgE-induced release of lipid mediators, the three different mast cell

populations analyzed exhibited large differences in their responses. Thus, in the cells used for the experiments shown in Fig. 8, preincubation with IgE alone had a substantial enhancing effect on the ability of cells to release LTC₄ in response to anti-IgE at 1 μg/ml. By contrast, in the experiments shown in Fig. 9, cells preincubated with or without IgE released very small, and quite similar, amounts of LTC₄ in response to anti-IgE (at either 1 or 10 μg/ml).

Moreover, the variation in the ability of different batches of mast cells to produce mediators in response to anti-IgE challenge appeared to be greater in the case of some products than others. For example, the cells examined in Figs. 8 and 9 were much more similar in their ability to release PGD₂ in response to challenge with anti-IgE at 1 μg/ml than they were in their ability to produce LTC₄ under the same conditions.

Such differences in responses may reflect differences in baseline levels of FcεRI surface expression (in control cells that were not preincubated with IgE or IL-4) and/or multiple other factors.

Notably, there was less batch-to-batch variation in the responses of these mast cell populations to the effects of preincubation with IgE, IL-4, and/or IL-4 on anti-IgE-induced histamine release. In each of the five experiments of this type, the effects of preincubation with IgE or IL-4 alone were significantly less than those of preincubation with IgE plus IL-4. Moreover, we found that in high responder mast cells, defined as those that gave >10% specific histamine release upon anti-IgE challenge after preincubation for 4 days without IgE or IL-4, the effect of adding IL-4 to IgE for a 4-day preincubation was small, whether judged by levels of FcεRI expression or magnitude of anti-IgE-induced histamine release. By contrast, adding IL-4 to IgE for a 4-day preincubation generally resulted in substantially greater enhancement of FcεRI surface expression or anti-IgE-induced histamine release in the low responder cells (defined as those that gave <10% specific release of histamine upon anti-IgE challenge after preincubation for 4 days without IgE or IL-4).

Additional studies will be required to ascertain the mechanism(s) underlying the effects of IL-4 on mediator secretion by umbilical cord blood-derived human mast cells. Nevertheless, in the case of both histamine release and PGD₂ or LTC₄ production, the levels of anti-IgE-induced mediated release observed in cells preincubated with both IL-4 and IgE significantly exceeded those seen in cells preincubated with IgE alone.

Effects of IgE and/or IL-4 on FcεRI surface expression on differentiated mouse mast cells

A preliminary report indicated that, under certain circumstances, IL-4 can suppress FcεRI surface expression on mouse mast cells in vitro; however, the specific details of the conditions for testing IL-4 in these experiments were not described (29). To examine the effects of IL-4 preincubation on FcεRI surface expression on differentiated mouse mast cells, we first tested BALB/c BMCMCs, representative of relatively immature in vitro derived mouse mast cells, which had been preincubated for 4 days before flow cytometry with mouse IgE mAb (5 μg/ml), mouse rIL-4 (10 ng/ml), both IgE (5 μg/ml) and IL-4 (10 ng/ml), or neither IgE nor IL-4. As shown in Fig. 10, preincubation with IgE alone resulted in a marked increase in FcεRI expression in these cells (in confirmation of previous reports) (13, 14), whereas IL-4 had no significant effect, whether when used alone or in conjunction with IgE.

We then performed a similar experiment using more mature populations of mouse mast cells, freshly isolated peritoneal mast cells (PMCs). Because mouse mast cells themselves represent a potential source of IL-4 (5, 6, 13), we analyzed PMCs isolated from BALB/c IL-4^{-/-} mice (28) and from the corresponding wild-type (BALB/c IL-4^{+/+}) mice. In confirmation of the results of

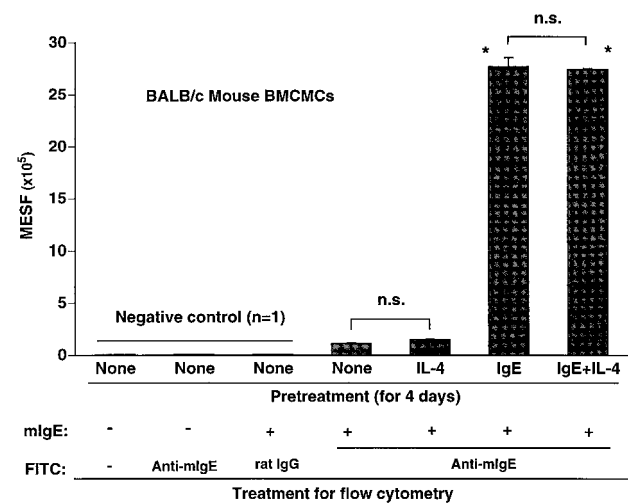


FIGURE 10. Surface FcεRI expression on BALB/c BMCMCs (cultured for 5 wk, purity >99%) cultured for 4 days with or without IgE (at 5 μg/ml) or IL-4 (at 10 ng/ml). For flow cytometry analysis of surface IgE binding, cells were briefly incubated with excess IgE and stained with FITC anti-IgE as in Fig. 1; data shown are mean ± SEM (*n* = 3). Negative controls were as described in Fig. 1B. *, *p* < 0.0001 versus values for mast cells that had been cultured for 4 days without IgE or IL-4.

Banks and Coleman (30), we found that PMCs derived from IL-4^{-/-} mice exhibited lower FcεRI surface expression at baseline (MESF = 0.55 ± 0.04) than did PMCs from IL-4^{+/+} mice (1.87 ± 0.25, *p* < 0.001 versus values for IL-4^{-/-} PMCs) (Fig. 11). This finding probably reflected the lower levels of circulating IgE in the IL-4^{-/-} mice (13). Indeed, in mice that had been injected i.v. with mouse IgE mAb (100 μg/mouse, i.v., for each of 4 days before recovery of PMCs for flow cytometry) (13), MESF values for FcεRI surface expression by PMCs were statistically indistinguishable in IL-4^{-/-} versus IL-4^{+/+} PMCs (1.84 ± 0.09, *n* = 4 versus 1.93 ± 0.07, *n* = 5; *p* > 0.05).

Incubation with IgE in vitro resulted in substantial enhancement of FcεRI surface expression in both IL-4^{-/-} and IL-4^{+/+} PMCs, an effect that, in the case of the IL-4^{-/-} PMCs, must have been entirely independent of IL-4. By contrast, incubation with exogenous IL-4 alone had little or no effect on FcεRI surface expression. However, for PMCs derived from either IL-4^{+/+} or IL-4^{-/-} mice, MESF values for FcεRI surface expression were significantly

higher in cells that had been incubated with IL-4 and IgE than in those that had been incubated with IgE alone.

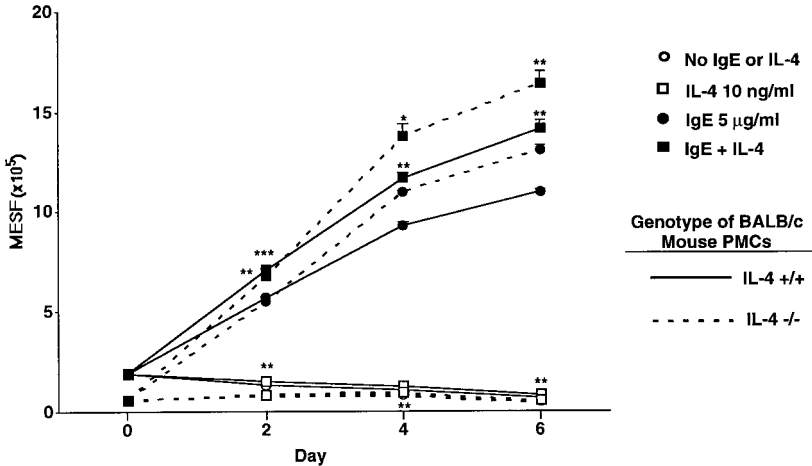
Discussion

We previously reported that exposure to monomeric IgE can significantly up-regulate the ability of human umbilical cord blood-derived mast cells to bind IgE to their surface (18). In the present study, we formally demonstrate that this result largely, if not solely, reflects the ability of IgE to induce increased surface expression of FcεRI. In confirmation of prior studies (20–22), we also found that, in some experiments, incubation of human mast cells with IL-4 resulted in a modest enhancement of their surface expression of FcεRI. However, in our in vitro derived human mast cell preparations, unlike the human fetal liver-derived mast cells in earlier stages of development that were studied by Xia et al. (22), monomeric IgE exhibited a much more striking ability to up-regulate mast cell surface expression of FcεRI than did IL-4 alone. We obtained very similar findings with two different populations of differentiated mouse mast cells, in vitro derived BMCMCs and in vivo derived PMCs. On the other hand, IL-4 and IgE acted synergistically in umbilical cord blood-derived human mast cell populations (and in mouse peritoneal mast cells) to enhance FcεRI surface expression, findings that are consistent with the results reported by Xia et al. (22) for fetal liver-derived human mast cells.

The differences between some of our results and those of Xia et al. (22) may largely reflect differences in the mast cells that were analyzed in the two studies. We performed our experiments comparing the effects of IgE and IL-4 on FcεRI expression using differentiated mast cells that were obtained by maintaining umbilical cord blood mononuclear cells for at least 9 wk in medium supplemented with SCF, IL-6, and PGE₂ (24). By contrast, the recent report by Xia et al. (22) (which was published after many of the experiments reported herein had been completed) utilized fetal liver-derived human mast cells that were generated in SCF-containing medium, but were investigated only during the first 4 wk of their development in vitro. Moreover, in the fetal liver-derived mast cell system, the effects of IL-4 on FcεRI expression were greatest when the cytokine was present during the earliest stages (i.e., the first 2 wk) of mast cell development in vitro (22).

Accordingly, one possible explanation for the differences between our results and those of Xia et al. (22) is that, in mast cells that have undergone more extensive differentiation/maturation in vitro, FcεRI expression becomes less responsive to IL-4 and more

FIGURE 11. Time course of changes in surface FcεRI expression on peritoneal mast cells from BALB/c IL-4^{+/+} or IL-4^{-/-} mice cultured with or without IgE and/or IL-4. Peritoneal cells were cultured with or without IgE (at 5 μg/ml) or IL-4 (at 10 ng/ml). Surface FcεRI expression was analyzed by flow cytometry. All data are mean ± SEM (*n* = 3). *, *p* < 0.05; **, *p* < 0.01; ***, *p* < 0.0001, versus MESF values of corresponding cells that had been maintained at the same concentration of IgE (i.e., 0 or 5 μg/ml), but without IL-4 in the same genotype of mice (i.e., IL-4^{+/+} or IL-4^{-/-}).



responsive to IgE. If this hypothesis is correct, it raises the possibility that the same may be true in vivo, i.e., that IL-4 is important in promoting FcεRI expression during early stages of mast cell development, through either direct or indirect effects on mast cells, but that IgE becomes more important than IL-4 as a regulator of FcεRI expression at later stages of mast cell differentiation/maturation. Alternatively, some of the differences obtained in the two studies may reflect intrinsic differences between umbilical cord blood-derived as opposed to fetal liver-derived human mast cells.

The effects of IL-4 on FcεRI expression may represent one component of a wide spectrum of actions of this cytokine (when tested in concentrations similar or identical to those used in this study) on mast cell development and function. For example, in human umbilical cord blood-derived mast cells generated in medium containing rhSCF and rhIL-6, exposure of the cells to IL-4 at 10 ng/ml induced morphologic changes consistent with enhanced mast cell maturation, and also increased the cells' expression of chymase, changes that were apparent as early as 5 days after the first exposure to exogenous IL-4 (31). In mouse mast cells, exposure to IL-4 not only enhanced the ability of cells to release serotonin in response to challenge with IgE and specific Ag, but also increased the serotonin release of the cells in response to challenge with compound A23187 (32). Exposure to IL-4 also can enhance the ability of in vitro derived mouse mast cells to release histamine and LTC₄ in response to endothelin-1 (33).

It seems very unlikely that the effects of IL-4 on mast cell secretion of mediators in response to compound A23187 (this study and Ref. 32) or endothelin-1 (33) would be due to any effects of the cytokine on surface expression of FcεRI. Indeed, in the present study, it is possible that the enhanced anti-IgE-induced release of histamine and lipid mediators observed in cells that had been preincubated with IL-4 and IgE, as opposed to IgE alone (Figs. 8 and 9), reflected a combination of the effects of IL-4 exposure on both FcεRI surface expression and on other, FcεRI-independent, aspects of mast cell maturation and secretory function.

While the precise mechanisms underlying the spectrum of effects of IL-4 on human mast cell mediator secretion remain to be fully elucidated, increasing evidence indicates that exposure to monomeric IgE can enhance IgE-dependent mast cell mediator release largely, if not entirely, by up-regulating levels of surface expression of the FcεRI. Thus, IgE-dependent up-regulation of human mast cell FcεRI expression can significantly enhance the sensitivity and/or intensity of the response of cells to anti-IgE challenge, as reflected by the secretion of increased quantities of histamine (this study and Ref. 18), LTC₄ and PGD₂ (this study), MIP-1α (18), or tryptase (22). Moreover, our preliminary studies indicate that IgE-dependent up-regulation of FcεRI surface expression can also enhance the ability of umbilical cord blood-derived human mast cells to secrete IL-13 (our unpublished data).

Our findings suggest that any mechanism that results in the substantial elevation of IgE levels is most likely also to result in significantly enhanced IgE-dependent human mast cell function. IgE can also regulate surface FcεRI expression by human (34) or mouse (35) basophils, raising the possibility that circulating or local concentrations of IgE can enhance FcεRI expression, and FcεRI-dependent function, in other cell types as well. For example, it will be of interest to determine whether this mechanism can account, at least in part, for observations indicating that subjects with atopy may express increased levels of FcεRI on CD1a⁺ Langerhans cells (36) or circulating monocytes (37), or for the apparent differences in the levels of FcεRI expression in various populations of eosinophils (38). Furthermore, no matter what minimum number of cross-linked FcεRI may be required to elicit functional responses in FcεRI⁺ effector cells (39, 40), cells that express in-

creased capacity for IgE binding on their surface can be sensitized adequately with larger numbers of different IgE species of distinct Ag specificities.

Our findings strongly support the hypothesis that approaches that significantly diminish circulating levels of IgE have the potential to reduce the IgE-binding capacity of tissue mast cells, as well as circulating basophils (34, 41), and that this, in turn, can diminish IgE- and FcεRI-dependent mast cell effector function. Indeed, this represents one of the more likely explanations for the observation that subjects in whom serum levels of IgE had been markedly reduced as a result of treatment with an anti-human IgE Ab also exhibited diminished wheal and flare reactions in response to intradermal allergen challenge (34).

Finally, we found that individual batches of umbilical cord blood-derived human mast cells exhibited wide variation in their ability to release histamine in response to anti-IgE challenge, particularly if the cells had not been preincubated with IgE for 4 days before further sensitization with IgE just before challenge with anti-IgE. Based on the results shown in Figs. 8 and 9, it appears that mast cells may also exhibit considerable variation in their ability to release lipid mediators in response to anti-IgE challenge. By contrast, less variation was observed in the ability of different populations of mast cells to undergo up-regulation of FcεRI surface expression in response to preincubation for 4 days with IgE.

Because each cord blood preparation was derived from a genetically distinct, albeit unidentified, donor, the variation in the responses of different populations of umbilical cord blood-derived mast cells to anti-IgE challenge may have reflected genetic differences in the donors. It is even possible that some of these genetic differences may also influence the susceptibility of these individuals to the development of allergic diseases. However, there are many other factors that may have contributed to these results, including differences in the responses of individual cord blood cell preparations to our conditions of culture.

While extensive further studies may be needed to understand the basis for these observations, the potential variability of the responses of different cord blood-derived human mast cell populations to challenge with anti-IgE should be kept in mind whenever the results obtained with such mast cell populations are analyzed.

Acknowledgments

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